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Blood Flow Velocity in Cutaneous Lesions of Leprosy[1]

Indropo Agusni, J. Swanson Beck, Robert C. Potts,
Ian A. Cree, and M. Ibeni Ilias[2]

Leprosy shows great variation in its clinical and pathological manifestations, both between patients and within a particular patient, during the course of the disease. The features of the disease are generally believed to be related to the position of the patient within the immunopathological spectrum and this, in turn, is based on the extent of cell-mediated immunity (CMI) to *Mycobacterium leprae* ([10, 13]). However, within each type of disease recognized in the immunopathological spectrum (e.g., TT-BT) there may be considerable variation in the extent and clinical character of the skin involvement. The extent can clearly be recorded serially by mapping techniques, but objective measurement of the severity of the lesions is currently dependent on biopsy and so frequent reassessments of these characteristics are not practical in clinical surveys.

The main tissue response to infection with *M. leprae* is an immunologically mediated, chronic inflammation with well-demarcated foci of granuloma in the dermis and relatively little damage to the intervening tissue. From a biopsy it is possible to grade the extent of skin involvement by measurement of the "granuloma fraction" (GF) either by observer grading on direct microscopy ([11]) or more precisely by semi-automated histometry ([5]): the density of bacilli in the lesion can be assessed by direct microscopy and graded as the "Bacillary Index" ([12]).

Recently, a technique has been developed for noninvasive measurement of skin blood flow in the microcirculation based on detection of the extent of change in wavelength in light reflected from moving particles (mainly erythrocytes in the skin blood vessels), and this has been validated against established methods such as isotope clearance ([6]). The laser-Doppler velocimetry technique can be used to measure blood flow changes during the course of the tuberculin skin test accurately and precisely with no pain or disturbance to the patient on repeated examination ([1, 2]).

This paper describes the application of the laser-Doppler technique for the measurement of blood flow in the cutaneous lesions of leprosy, and relates the microcirculatory changes observed to the clinical appearance and histopathology of the lesions.

MATERIALS AND METHODS

Patients. The study was performed in the Dermatology Clinic at Dr. Soetomo General Hospital, Surabaya, Indonesia, on male patients (aged 11–58 years) living in Surabaya and adjacent parts of East Java. All were under treatment (1 to 8 months) with the standard World Health Organization (WHO) multidrug (MDT) regimen; four were graded borderline tuberculoid (BT) on the Ridley-Jopling scale and five as borderline lepromatous/lepromatous (BL/LL), all with histopathological confirmation of the diagnosis. One BT and one BL patient were recognized clinically as being in reversal reaction. Three BL patients were re-examined after 3 to 7 days.

Laser-Doppler measurement of skin blood flow velocity. Before measurement the patient adopted a comfortable posture appropriate to the area being examined to minimize movement during the measurements. Blood flow was measured with a laser-Doppler flowmeter (Model PF2, Perimed,

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[2] I. Agusni, M.D., Lecturer in Dermatology, and M. I. Ilias, M.D., Professor of Dermatology, Airlangga University, Surabaya, Indonesia. J. S. Beck, M.D., F.R.C.P., F.R.C.Path., F.I.Biol., F.R.S.E., Professor of Pathology; R. C. Potts, F.I.M.L.S., Chief Medical Laboratory Scientific Officer; I. A. Cree, B.M.Sc., M.B., Ch.B., Ph.D., Lecturer in Pathology, University of Dundee, Ninewells Hospital and Medical School, P.O. Box 120, Dundee DD1 9SY, Scotland, U.K.

Reprint requests to Professor J. Swanson Beck.

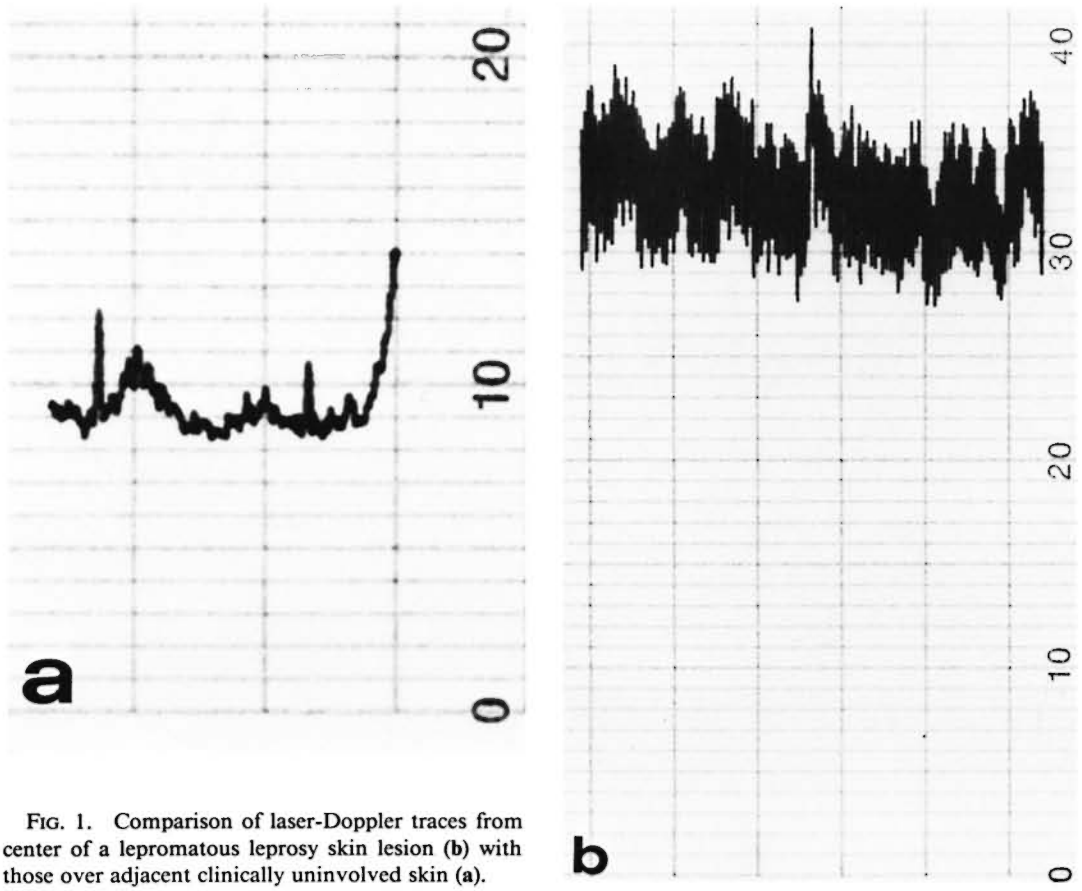

FIG. 1. Comparison of laser-Doppler traces from center of a lepromatous leprosy skin lesion (**b**) with those over adjacent clinically uninvolved skin (**a**).

Stockholm, Sweden). A probe holder was attached to the skin at the selected site with double-sided adhesive tape to align the fiber-optics in optimal position. The principles governing the measurement of skin blood flow by the method are well established ([8]). Essentially, coherent light from a 2 mW helium-neon laser is brought to the skin by an optical fiber and undergoes multiple scattering and absorption in a small hemispherical volume of tissue with a radius of approximately 1 mm ([7, 9]). When light is reflected from a moving object, such as an erythrocyte (RBC) in blood flowing through a capillary, the wavelength will be Doppler-shifted. Some of the incident illumination will be directed out of the tissue and is picked up by the efferent optical fibers in the head of the probe and taken back to the instrument for signal processing. The Doppler-shifted part of the signal is isolated and converted into a voltage output signal which is linearly related to the RBC flux (defined as the number of RBC in the measured volume times their mean velocity) and this was recorded with a pen recorder. The signal has two forms of periodicity (related to the arterial pulse and vasomotion, respectively) and its intensity is measured as the mean RBC flux ([2]). The appearance of typical signals from normal and inflamed skin is shown in Figure 1. Recordings were made at each site for 1 to 2 min, and the mean DC voltage of each trace was estimated from the mean of the peak-to-trough fluctuations in the oscillatory signal. The measurements of pulse amplitude and vasomotion amplitude in both tuberculoid and lepromatous patients were always related proportionately to the RBC flux signals and, therefore, these observations were not analyzed separately.

Measurements were made at several points in the center and at the edge of each lesion and from adjacent uninvolved skin. The location of each observation was

FIG. 2. Clinical photograph of a BT lesion of 1-month duration on lower outer aspect of right arm of a 15-year-old boy (patient 1, lesion labeled 51). Points at which laser-Doppler measurements were made are indicated by the felt pen marks.

marked on the skin with a felt pen for photographic recording (Fig. 2). When the patient had multiple lesions, several were selected for measurement. The RBC flux of uninvolved skin was much greater over the face than over the trunk and extremities where there was less regional anatomical variation. Accordingly, for convenience of analysis, a record was made of the increase in RBC flux at each measured point over the basal level in uninvolved skin nearby.

Biopsy of leprosy lesions. Biopsies of the lesions were taken under 2% plain lignocaine local anesthesia with a 4-mm disposable skin punch (Stiefel Laboratories Ltd., Slough, England) from four BL/LL patients at sites of prior laser-Doppler measurement. All subjects gave informed permission without coercion. The core of tissue was snap-frozen in an acetone-dry ice slurry. Upon return to Dundee, each tissue block was cryopreserved in glycerol ([4]) before 5 μm cryostat sections were cut. The sections were stained with hemalum (hematoxylin and alum) and eosin for confirmation of the diagnosis of leprosy and placement within the immunopathological scale ([13]). The granuloma fraction was measured by microprocessor-based semi-automated planimetry ([5]), and the bacillary index was determined by direct microscopy ([12]).

RESULTS

Laser-Doppler measurement of skin blood flow

Tuberculoid patients. Three of the tuberculoid leprosy patients had flat hypopigmented plaques with clearly demarcated

TABLE 1. *Laser-Doppler measurements (RBC flux) over cutaneous leprosy lesions.*

Patient[a] no.	Age	Lesion site	Leprosy type	Increase in RBC flux over lesion compared with adjacent uninvolved skin			
				Edge of plaque		Center of plaque	
				No. sites measured	Mean increase in signal (volts)	No. sites measured	Mean increase in signal (volts)
1	20	Arm/back	BT	6	0.35	6	0.3
2	30	Shoulder/abdomen	BT	3	0.4	4	0.35
3	28	Face	BT	4	1.5	—	—
4	24	Back/shoulder	BT[b]	6	10.5	4	1.3
5	11	Arm	BL/BB	10	1.8	11	3.8
		Back		10	0.4	10	2.2
6	58	Shoulder	BL	3	1.4	3	8.9
7	47	Back	BL	3	0.8	3	9.2
8	24	Back/buttock	Idt[c]	—	—	5	0.8
9	35	Arm	BL[b]	4	0.9	6	4.7

[a] All patients were males.
[b] Patient in reversal reaction.
[c] Idt = indeterminate.

TABLE 2. *Summary of histometric findings.*

Patient no.	Biopsy site	Leprosy type[a]	BI[b]	GF[c]
5	Back nodule 61	LL	1+	69.8
	Back nodule 62	BL	1+	59.9
6	R. side	BL	4+	50.4
	Back	LL/BL	3+	78.2
7	L. forearm	LLs	3+	87.6
	R. forearm	BL	2+	81.1
8	Back	Idt	1+	17.0
	L. buttock	LLs	3+	62.9

[a] LL = lepromatous leprosy; BL = borderline lepromatous; Idt = indeterminate; LLs = subpolar lepromatous.
[b] BI = bacterial index.
[c] GF = granuloma fraction.

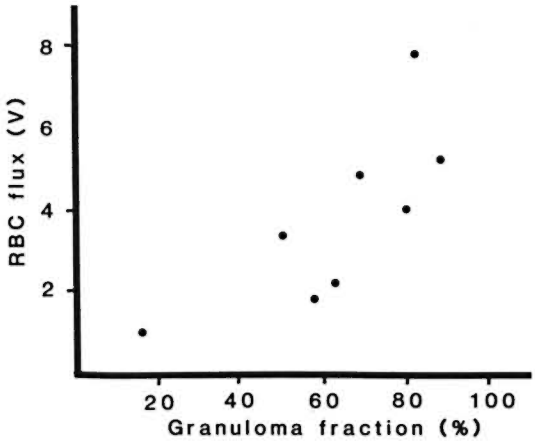

FIG. 3. Graph relating laser-Doppler measurements to granuloma fractions in four LL-BL patients. The magnitude of the laser-Doppler signal is a good indicator of the size of the granuloma fraction.

edges; they did not show skin thickening. The blood-flow velocity was measured in each lesion usually over three sites at the edge and another three sites in the center and comparative measurements were made in adjacent uninvolved skin. The blood-flow velocity was substantially greater (3.3-fold) in the normal facial skin (mean value 2.9 V, patient 3) than in other areas of the body (mean value 0.87 V, patients 1 and 2). The results of measurements over tuberculoid leprosy skin lesions are summarized in Table 1. The blood-flow velocity over the lesions was slightly raised above that in the normal skin (95% confidence limits for difference in means 0.05 to 0.59 V; $t = 2.46$; $p < 0.05$), but the readings over the edge were not significantly different from those over the center of the plaque. However, the extent of hyperemia in stable BT skin lesions was not great.

The tuberculoid patient in reaction (patient 4) showed raised, very erythematous skin with a somewhat scaly surface at the edges of the plaques and thinned, rather scarred skin at the center. Compared with normal skin, the blood flow was very markedly accelerated (up to 20-fold greater) at the edges of the plaques but only slightly raised (up to fourfold) in the center (Table 1). Generally, similar results were seen in the two lesions tested on this patient.

Lepromatous patients. The BL/LL patients had raised thickened skin in the lesions. In the four patients with stable disease, not in any reactional state, the larger lesions were sessile with gently rolled edges but the smaller lesions were often dome shaped with a convex surface and fairly sharply rising edges. The skin was often finely scaly over the center of the lesion but generally not hypopigmented. The lesions did not appear erythematous or feel hot. The blood-flow velocity was raised over the center of the lesions (generally 3 to 9 times greater than in the adjacent normal skin). The 95% confidence limits for the increase in the laser-Doppler signal over the adjacent uninvolved skin was 3.1 to 6.2 V ($t = 6.06$; $p < 0.0001$). The signal was not easy to record at the edge (particularly over the smaller lesions), since the probe holder would not adhere properly to the convex surface, but the level recorded was not much greater than that over the normal skin (Table 1). Three patients were retested after several days; similar readings were recorded when the sites were re-examined.

The fifth BL patient was in reversal reaction. The lesions were generally similar to those of the other BL patients. The signal level was also similar to the other BL/LL patients at the edge and center of the plaques (Table 1).

Measurement of GF by histometry

The punch biopsies were taken from the centers of two separate lesions from each of the four BL/LL patients not in reaction. The

results of the histometric studies are summarized in Table 2. The granuloma fractions in the sections from these eight lesions varied from 17% to 88%, and were clearly related to the mean laser-Doppler signal measured at the same sites immediately before each biopsy was taken (Fig. 3).

It was apparent on histological examination that the blood capillary density was high in all of the granuloma foci in the four pairs of biopsies examined. The density of blood capillaries was very much lower in the intervening dermis. The lesions from all of the patients appeared to be similar with respect to the contrast between the high capillary density within the granulomas and the low density of small blood vessels in the intervening uninvolved tissue.

DISCUSSION

This study has shown that the laser-Doppler method can be used successfully to measure dermal blood flow in the lesions of leprosy. The measurements are simple to perform, do not hurt or inconvenience the patient, and the results are reproducible. The procedure can be repeated as frequently as the observer wishes without upsetting the patient. The intensity of the laser-Doppler signal was clearly related to the size of the granuloma fraction in the underlying leprosy lesion. The small blood vessels that form the metabolic exchange microcirculation (capillaries and venules) are clearly more numerous in the granulomas than in the intervening dermis. These vessels have a relatively large lumen so it is likely that they will offer a low resistance to blood flow relative to that in the residual normal cutaneous microcirculation. Unfortunately, it was not possible to measure the density of the capillaries and venules in the granuloma histometrically, since these small vessels were frequently collapsed in the cryostat sections of snap-frozen biopsies. Nevertheless, it seems probable that the accelerated blood flow in the more extensive microcirculation in the granulomas (compared with that in the normal dermis) is the basis for the raised laser-Doppler signal over the lesions compared with that over noninvolved skin.

In general, the laser-Doppler signal was stronger over stable lepromatous lesions than over stable tuberculoid lesions; the explanation is clearly related to the greater granuloma fraction in the lepromatous form of the disease. Moreover, the edge of the tuberculoid lesion in reaction showed a great acceleration of blood flow, again in keeping with the histopathological changes. The acceleration of blood flow was even greater in lesions with reversal reactions. These changes may be related to the release of vasoactive mediators within the lesion, either from macrophages or possibly from mast cells ([3]).

Increased blood flow within leprosy skin lesions is assessed subjectively as erythema during clinical examination. Thermography could be used to provide a more objective assessment, but the equipment required is delicate and requires a temperature-controlled environment. The laser-Doppler equipment is both robust and portable. Since erythema may be an early sign of reversal reaction in leprosy lesions, the laser-Doppler technique may prove useful for an objective noninvasive assessment in the early diagnosis of reactions during chemotherapy trials in leprosy patients.

SUMMARY

The velocity of blood flow in the cutaneous lesions of leprosy was measured by the noninvasive technique of laser-Doppler velocimetry in nine male patients, all under treatment with World Health Organization multidrug regimens. In three patients with stable borderline tuberculoid (BT) lesions, the blood-flow velocity was slightly faster over the lesion than that in adjacent uninvolved skin. There was no substantial difference between different sites at the center and edge of individual lesions in each patient, but there was some variation between subjects. In one other BT patient with a reversal reaction, blood-flow velocity was 20-fold greater than in adjacent uninvolved skin. In four patients with stable borderline lepromatous/lepromatous (BL/LL) disease, the blood-flow velocity was 3 to 9 times faster over the plaques than in adjacent skin. There was relatively little difference between measurements over comparable points at the edge and center of individual plaques, or between plaques in the same patient, but there were considerable differ-

ences between patients. A fifth BL patient in reversal reaction showed generally similar results to those found in the stable BL/LL patients.

Histometric study of the biopsies taken immediately after blood-flow measurement from two different plaques on each of four BL patients showed a clear relationship between the granuloma fraction measured by planimetry and the blood-flow velocity. This finding suggests that laser-Doppler velocimetry might prove to be a useful, clinically acceptable, noninvasive technique for monitoring the severity of hyperemia as an early indication of reversal reaction during chemotherapy trials in leprosy patients.

RESUMEN

Se midió la velocidad del flujo sanguíneo en las lesiones cutáneas de la lepra por la técnica de velocimetría laser-Doppler en 9 pacientes masculinos en tratamiento con el esquema de drogas múltiples propuesto por la Organización Mundial de la Salud. En 3 pacientes con lesiones tuberculoides limítrofes (BT) estables, la velocidad de flujo sanguíneo fue ligeramente más rápida sobre la lesión que sobre la piel adyacente no afectada. No hubo una diferencia substancial entre diferentes sitios del centro y del borde de lesiones individuales en cada paciente pero hubo cierta variación entre pacientes. En otro paciente BT con reacción reversa, la velocidad de flujo sanguíneo fue 20 veces más grande que en la piel adyacente no afectada. En 4 pacientes con lepra lepromatosa subpolar (BL/LL) estable, la velocidad de flujo fue de 3 a 9 veces más rápida sobre las placas que en la piel adyacente. Hubo relativamente poca diferencia entre mediciones sobre puntos comparables en el borde y en el centro de placas individuales o entre placas en el mismo paciente pero hubieron diferencias considerables entre pacientes. Un quinto paciente BL en reacción reversa mostró generalmente resultados similares a los encontrados en pacientes BL/LL estables.

El estudio histométrico de las biopsias tomadas inmediatamente después de la medición del flujo sanguíneo en dos placas diferentes de cada uno de 4 pacientes BL, mostró una clara relación entre la fracción granuloma medida por planimetría y la velocidad de flujo sanguíneo. Este hallazgo sugiere que la velocimetría de laser-Doppler puede ser una técnica no invasiva, clínicamente aceptable, útil para valorar la severidad de la hiperhemia como índice temprano de reacción reversa durante los programas de quimioterapia en los pacientes con lepra.

RÉSUMÉ

Au moyen d'une technique non envahissante de vélocimétrie par la méthode laser-Dopler, on a mesuré la vitesse du flux sanguin dans les lésions cutanées de la lèpre, chez neuf malades de sexe masculin, qui tous étaient traités par les posologies de polychimiothérapie recommandées par l'Organisation Mondiale de la Santé. Chez trois malades présentant des lésions stables de lèpre tuberculoïde dimorphe (BT), la vitesse du flux sanguin était légèrement plus rapide dans la lésion que dans les régions adjacentes de la peau qui n'étaient pas atteintes de lésions. On n'a pas noté de différences notables entre les différents endroits de la lésion, soit au centre ou aux bords des lésions individuelles, chez aucun des malades, mais on a cependant noté quelques variations parmi les différents sujets. Chez un autre malades atteint de lèpre BT, avec réaction reverse, la vitesse du flux sanguin était vingt fois plus rapide que dans les régions adjacentes de la peau non affectée. Chez quatre malades souffrant de lèpre lépromateuse dimorphe stable, ou de lèpre lépromateuse (BL/LL), la vitesse du flux sanguin était trois à neuf fois plus rapide à la surface des plaques que dans la peau adjacente. On a relevé relativement peu de différences entre les mesures pratiquées respectivement à la surface de points situés aux bords et au centre des plaques individuelles, ou entre les plaques mesurées chez le même malade. Par contre, les différences étaient considérables d'un malade à l'autre. Un cinquième malade atteint de lèpre BL, avec réaction reverse, a montré dans l'ensemble des résultats semblables à ceux qui avaient été observés chez les malades souffrant de lèpre BL/LL stable.

L'étude histométrique des biopsies prélevées immédiatement après la mesure du flux sanguin au niveau de deux plaques différentes chez chacun des quatre malades BL, a montré une relation nette entre la fraction du granulome mesuré par planimétrie et la vitesse du flux sanguin. Cette observation suggère que la vélocimétrie mesurée par la méthode du laser-Dopler peut être une technique non envahissante, à la fois utile et cliniquement acceptable, pour évaluer la gravité de l'hyperhémie, en tant qu'indicateur précoce de la réaction reverse survenant au cours d'essais cliniques de chimiothérapie chez des malades de la lèpre.

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